Breed Distribution of *SOD1* Alleles Previously Associated with Canine Degenerative Myelopathy

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Background: Previous reports associated 2 mutant *SOD1* alleles (*SOD1:c.118A* and *SOD1:c.52T*) with degenerative myelopathy in 6 canine breeds. The distribution of these alleles in other breeds has not been reported.

Objective: To describe the distribution of *SOD1:c.118A* and *SOD1:c.52T* in 222 breeds.

Animals: DNA from 33,747 dogs was genotyped at *SOD1:c.118*, *SOD1:c.52*, or both. Spinal cord sections from 249 of these dogs were examined.

Methods: Retrospective analysis of 35,359 previously determined genotypes at *SOD1:c.118G > A* or *SOD1:c.52A > T* and prospective survey to update the clinical status of a subset of dogs from which samples were obtained with a relatively low ascertainment bias.

Results: The *SOD1:c.118A* allele was found in cross-bred dogs and in 124 different canine breeds whereas the *SOD1:c.52T* allele was only found in Bernese Mountain Dogs. Most of the dogs with histopathologically confirmed degenerative myelopathy were *SOD1:c.118A* homozygotes, but 8 dogs with histopathologically confirmed degenerative myelopathy were *SOD1:c.118A/G* heterozygotes and had no other sequence variants in their *SOD1* amino acid coding regions. The updated clinical conditions of dogs from which samples were obtained with a relatively low ascertainment bias suggest that *SOD1:c.118A* homozygotes are at a much higher risk of developing degenerative myelopathy than are *SOD1:c.118A/G* heterozygotes.

Conclusions and Clinical Importance: We conclude that the *SOD1:c.118A* allele is widespread and common among privately owned dogs whereas the *SOD1:c.52T* allele is rare and appears to be limited to Bernese Mountain Dogs. We also conclude that breeding to avoid the production of *SOD1:c.118A* homozygotes is a rational strategy.

Key words: Amyotrophic lateral sclerosis; Cytoplasmic aggregates; DNA test; Spinal cord.

Taken literally, the term "canine degenerative myelopathy" (DM) could refer to a wide variety neurologic diseases of dogs. Since 1973, however, the term DM most often has been used to describe a progressive neurodegenerative disease (or diseases) with characteristic clinical signs and distinct histopathologic spinal cord lesions.[1–13] Most dogs are at least 8 years old before the onset of clinical signs. The initial signs of DM typically include asymmetric general proprioceptive ataxia and spastic paresis in the pelvic limbs. At this stage, segmental spinal reflexes are indicative

Abbreviations:

ALS	amyotrophic lateral sclerosis
DM	degenerative myelopathy
SOD1	superoxide dismutase 1

of upper motor neuron loss. Disease progression is relentless, and dog owners often elect euthanasia within 1 year after onset of clinical signs when their dogs become paraplegic.[2] When euthanasia is delayed, weakness can ascend to the thoracic limbs with the emergence of lower motor neuron signs such as flaccid tetraplegia, widespread muscle atrophy, dysphagia, and inability to bark.[3–5] Because a variety of common acquired compressive spinal cord diseases can mimic the early signs of DM by compromising upper motor neuron pathways, a definitive diagnosis of DM can only be made postmortem by the histopathologic observation of axonal and myelin degeneration along with astrogliosis in spinal cord funiculi. These degenerative changes can occur throughout the spinal cord white matter, but usually are most severe in the dorsal portion of the lateral funiculus in the middle to lower thoracic spinal cord segments.[1,6–9] Other histopathologic lesions in dogs with DM can include denervation atrophy in muscle and nerve fiber loss with axonal degeneration and secondary demyelination.[3,10]

In 2009, we reported that dogs with histopathologically confirmed DM were homozygous for the *A* allele of a *SOD1* missense mutation, *SOD1:c.118G > A*, which predicts a p.E40K amino acid substitution in superoxide dismutase 1 (SOD1).[3] More recently, we described a histopathologically confirmed case of DM

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in a dog that tested homozygous for the ancestral *SOD1:c.118G* allele.[11] This dog was homozygous for the *T* allele of a different *SOD1* missense mutation, *c.52A > T*, which predicts a p.S18T amino acid substitution. The *SOD1:c.52T* homozygote was a Bernese Mountain Dog. The earlier study that identified the *SOD1:c.118G > A* mutation focused on 5 canine breeds: Boxer, Chesapeake Bay Retriever, German Shepherd Dog, Pembroke Welsh Corgi, and Rhodesian Ridgeback.[3] Nonetheless, members of many other canine breeds have been diagnosed with DM.[2,5,12,13] To provide a broader view of the potential contribution of the known *SOD1* missense mutations across multiple canine breeds, we here report 35,359 genotypes at *SOD1:c.118*, *SOD1:c.52*, or both determined using DNA samples from members of 222 canine breeds, including 249 dogs from which spinal cord segments were obtained post mortem for histopathologic evaluation.

Materials and Methods

This report includes the results of all *SOD1:c.118G > A* or *SOD1:c.52A > T* genotype determinations completed at the University of Missouri before April 5, 2013. These genotypes were generated by testing DNA from a variety of sources, including previously collected samples in the University of Missouri Animal DNA Repository or in the Canine Health Information Center DNA Bank (offa.org/chicdnabank.html). Other samples were submitted by dog owners or their veterinarians to the University of Missouri or to the Orthopedic Foundation for Animals for DNA testing for mutations associated with a variety of inherited disorders including DM, mostly for diagnostic purposes or to be used for marker-assisted mate selection. DNA was extracted from EDTA-anticoagulated blood samples as previously described.[3] Other samples were collected using buccal swabs[a] and stored on DNA storage cards.[a] DNA was extracted from 3.2 mm diameter disks punched from the cards into 96-well plates with an electric puncher.[b] After 5 washes with distilled water at room temperature, 40 μL of distilled water was added to each well that contained a disk and the 96-well plates were heated at 95°C for 30 minutes. The dissolved DNA samples were separated from the disks by transfer to another 96-well plate.

Genotypes at *SOD1:c.118G > A* were determined as previously described[3] or with a TaqMan allelic discrimination assay,[14] which used 5'-GTGGGCCTGTTGTGGGTATCA-3' with 5'-CAAACTGA TGGACGTGGAATCC-3' for the PCR primers and 5'-VIC-CTCG CCTTTAGTCAGC-MGB-3' (*A* allele) and 5'-FAM-CGCCTT CAGTCAGC-MCB-3' (*G* allele) for the competing probes. Genotypes at *SOD1:c.52A > T* also were determined with a TaqMan allelic discrimination assay. The PCR primer sequences were 5'-G CCGTGTGCGTGTTGAAG-3' with 5'-CCCGCCCTTGCCTTC T-3' and the probe sequences were 5'-VIC-AGTGGATGGTGC CCTC-MGB-3' for the *A* allele and 5'-FAM-TGGATGGAG CCCTC-MGB-3' for the *T* allele.

The resequencing of *SOD1* in DNA from individual dogs was accomplished by PCR amplification with primers flanking the coding regions and exon-intron junctions of all 5 *SOD1* exons. Table S1 contains the PCR primer sequences, the expected sizes of the amplicons, and the annealing temperatures used for these PCR amplifications. The PCR amplifications for exon 2 through exon 5 were done with a kit[c] and 40 cycles of 95°C for 30 seconds, the annealing temperature for 30 seconds, followed by an additional 30 seconds at 72°C. For exon 1, the PCR amplifications were done with a different kit[d] designed for use with G-C rich templates and 24 cycles of 95°C for 30 seconds, 64°C for 30 seconds, followed by an additional 30 seconds at 72°C. The PCR amplicons were purified with a spin column[e] and sequenced in both directions with an automated Sanger sequencer.[f] DNA sequence analysis software[g] was used to compare the nucleotide sequences from individual dogs to one another and to build 3.1 of the NCBI canine genome reference sequence.

All of the 249 canine thoracic spinal cords in this study were dissected at necropsy from dogs that were at least 8 years old. They were fixed and embedded in paraffin. Sections of the paraffin-embedded samples were stained with hematoxylin and eosin and with either luxol fast blue-periodic acid Schiff or luxol fast blue-cresyl violet to detect neuronal fiber and myelin loss as previously described.[3] Previously described procedures[3] were used to immunostain spinal cord sections for glial fibrillary acid protein to detect astrogliosis and for SOD1 to detect SOD1-containing cytoplasmic aggregates. The diagnosis of DM was confirmed by demonstration of the characteristic pattern of axonal degeneration, myelin loss, and gliosis in the thoracic spinal cord. All spinal cord sections were examined by the same board-certified veterinary pathologist (GCJ).

Results

Before April 5, 2013, DNA from 33,746 individual dogs had been genotyped for the DM-associated *SOD1:c.118G > A* mutation at the University of Missouri. This included 1,368 mixed-breed dogs and 32,378 dogs claimed by their owners to be purebred representatives of 222 different canine breeds. Almost half (49%) of these dogs (n = 16,550) were homozygous for the ancestral (or *G*) allele whereas 9,112 dogs (27%) were *A/G* heterozygotes and 8,084 dogs (24%) were homozygous for the mutant (or *A*) allele. The frequency of the *A* allele for all genotyped dogs was 37%. Table S2 shows the uneven distributions of *A* allele frequencies among all 222 of the genotyped canine breeds. The *A* allele was detected in at least 1 representative from 124 breeds. Table 1 shows the distribution of *A* allele frequencies in the subset of breeds with at least 50 genotyped representatives. Among the 65 breeds in this subset, the Wire Fox Terrier had the highest *SOD1:c.118A* allele frequency (94%) whereas for 9 of these breeds all representatives tested homozygous for the *c.118G* allele.

A total of 1,613 dogs were genotyped for the *SOD1:c.52A > T* mutation. All 701 tested mixed-breed dogs and members of 56 breeds other than the Bernese Mountain Dog were homozygous for the ancestral *c.52A* allele (see Table S3). Among the 912 genotyped Bernese Mountain Dogs, 59 were heterozygotes and 2 (including a previously described dog[11]) were homozygous for the *SOD1:c.52T* allele. Thus, the *SOD1:c.52T* allele frequency among the 912 genotyped Bernese Mountain Dogs was 3.5%. All but 1 of these Bernese Mountain Dogs also were genotyped at *SOD1:c.118*. Table 2 shows the distribution of genotypes among the 911 Bernese Mountain Dogs genotyped at both *SOD1* loci. Notable was the absence of dogs with any of the 3 genotypes (*c.52T/T* and *c.118A/A*; *c.52T/T* and *c.118G/A*; and, *c.52A/T* and *c.118A/A*) that require the presence of a haplotype consisting of both *c.52T* and *c.118A*. Because the *SOD1:c.52T* and *SOD1:c.118A*

Table 1.   Genotypes at *SOD1:c.118* and A allele frequencies for breeds with at least 50 tested members.

Breed	Number Tested	Genotype			A Allele Frequency	Breed	Number Tested	Genotype			A Allele Frequency
		G/G	G/A	A/A				G/G	G/A	A/A	
Wire Fox Terrier	79	1	7	71	0.94	Borzoi	787	548	209	30	0.17
Pembroke Welsh Corgi	3,209	220	898	2,091	0.79	English Springer Spaniel	127	97	17	13	0.17
Boxer	3,934	500	1,177	2,257	0.72	Border Collie	80	63	7	10	0.17
Cavalier King Charles Spaniel	73	10	27	36	0.68	French Bulldog	87	64	18	5	0.16
Pit Bull Terrier	53	23	6	24	0.51	Irish Setter	57	42	12	3	0.16
American Water Spaniel	91	22	46	23	0.51	Shiloh Shepherd	221	161	53	7	0.15
Australian Shepherd	113	57	20	36	0.41	Great Pyrenees	85	68	10	7	0.14
Collie	151	73	39	39	0.39	Saint Bernard	78	60	15	3	0.13
Soft Coated Wheaten Terrier	88	45	18	25	0.39	Chinese Crested	53	40	13	0	0.12
Hovawart	64	31	17	16	0.38	Staffordshire Bull Terrier	52	46	1	5	0.11
Bernese Mountain Dog	2,413	941	1,112	360	0.38	Mastiff (English Mastiff)	114	93	19	2	0.10
Chesapeake Bay Retriever	2,344	958	1,034	352	0.37	Norwich Terrier	74	60	14	0	0.09
German Shepherd Dog	6,458	3,155	1,884	1,419	0.37	Australian Cattle Dog	61	53	5	3	0.09
Tibetan Terrier	69	32	24	13	0.36	Coton de Tulear	110	94	16	0	0.07
Kerry Blue Terrier	558	254	223	81	0.34	Labrador Retriever	475	433	19	23	0.07
Czechoslovakian Wolfdog	52	24	21	7	0.34	Nova Scotia Duck Tolling Ret.	59	52	6	1	0.07
Cardigan Welsh Corgi	544	271	194	79	0.32	Poodle-Standard	533	468	60	5	0.07
Pug	382	202	113	67	0.32	Beagle	57	52	3	2	0.06
Jack Russell Terrier	60	36	10	14	0.32	Belgian Malinois	101	92	6	3	0.06
Bloodhound	264	125	117	22	0.30	Tenterfield Terrier	66	59	7	0	0.05
Canaan Dog	169	87	67	15	0.29	Siberian Husky	97	92	3	2	0.04
Rhodesian Ridgeback	2,645	1,381	1,023	241	0.28	Dalmatian	180	172	4	4	0.03
Kuvasz	75	46	19	10	0.26	Golden Retriever	334	322	2	10	0.03
Welsh Terrier	72	41	25	6	0.26	Rottweiler	76	73	2	1	0.03
Komondor	55	32	22	1	0.22	Doberman Pinscher	55	54	0	1	0.02
Shetland Sheepdog	58	41	10	7	0.21	Alaskan Malamute	61	60	0	1	0.02
Tamaskan (Aatu Tamaskan)	59	40	17	2	0.18	Newfoundland	62	61	0	1	0.02
Puli	123	87	29	7	0.17	Rat Terrier	63	62	1	0	0.01

Breed (and number tested) for 9 breeds represented only by G/G homozygotes: Weimaraner (112); Great Dane (84); Field Spaniel (75); Basenji (59); Akita (58); English Cocker Spaniel (56); Greater Swiss Mountain Dog (53); Bouvier des Flandres (51); Dachshund (50).

Table 2.   Distribution of combined SOD1:c.52 and SOD1:c.118 genotypes in Bernese Mountain Dogs.

SOD1:c.52 Genotypes	*SOD1:c118* Genotypes		
	G/G	G/A	A/A
A/A	316	399	136
A/T	35	24	0
T/T	2	0	0

alleles rarely, if ever, occur together as a haplotype, the 24 dogs that are heterozygous at both c.52 and c.118 are most likely compound heterozygotes. Eleven of the 24 compound heterozygous Bernese Mountain

Dogs were >8 years old in May 2013 and we were able to ascertain the clinical status of 6 of them. One of them died from unrelated causes at 9.0 years of age. Another 1 was unaffected at 8.3 years of age. The other 4 compound heterozygous Bernese Mountain Dogs have exhibited clinical signs of DM, including 1 that was euthanized because of advanced disease. The histopathologic findings for this dog confirmed the diagnosis of DM.

Conclusions from histopathologic examination of 249 spinal cords from mixed-breed dogs and purebred dogs from 38 different breeds, including 213 dogs which previously had received a presumptive diagnosis of DM, are summarized in Table S4. In this table (and

in Table 3), the 45 spinal cords from dogs for which a presumptive diagnosis of DM was not supported by the histopathologic findings were grouped with the 36 spinal cords from dogs euthanized for reasons unrelated to DM and classified as controls. Of the 168 spinal cords with a confirmed diagnosis of DM, 157 were from SOD1:118A homozygous dogs, 9 (2 Bernese Mountain Dogs, 2 Chesapeake Bay Retrievers, 2 German Shepherd Dogs, 2 Rhodesian Ridgebacks, and 1 Alaskan Husky) were from SOD1:c.118A/G heterozygotes, and 2 were from SOD1:c.118G homozygotes. One of the 2 SOD1:118A/G heterozygous Bernese Mountain Dogs was a c.118A/G plus c.52A/T compound heterozygote as discussed above. The coding regions in all 5 SOD1 exons from the other 8 SOD1: c.118A/G heterozygotes with confirmed DM were analyzed by PCR amplification and automated Sanger sequencing, but no additional missense mutations were found. One of the 2 SOD1:118G homozygotes with confirmed DM was a previously described Bernese Mountain Dog that was homozygous for the T allele at SOD1:c.52[11] and other was from a 11-year-old German Shepherd Dog with a slightly slower rate of disease progression and a more pronounced sensorimotor neuropathy than is typical for DM. No potentially causal SOD1 sequence variants were found in DNA from this dog.

One hundred and seventy-three spinal cords, including 115 with confirmed DM and 58 controls, were examined by immunohistochemistry with anti-SOD1 antibodies. The results are summarized in Table 3 and photomicrographs from selected cases are shown in Fig S1. Aggregates containing SOD1 antigen were detected in all 105 spinal cords from SOD1:c.118A homozygous dogs with confirmed DM. In addition, the SOD1:c.52T homozygous Bernese Mountain Dog and all 8 of the examined SOD1:c.118A/G heterozygous dogs with histopathologically confirmed DM had SOD1 aggregates whereas no SOD1 aggregates were detected in the SOD1:c.118G homozygous German Shepherd Dog with confirmed DM. Among the 58 control spinal cords examined by immunohistochemistry,

Table 3. Results of immunohistochemical staining for SOD1 aggregates.

Breed	Number Tested	Confirmed DM						Controls					
		G/G		G/A		A/A		G/G		G/A		A/A	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Alaskan Husky	1	0	0	1	0	0	0	0	0	0	0	0	0
American Eskimo Dog	1	0	0	0	0	1	0	0	0	0	0	0	0
American Staffordshire Terrier	1	0	0	0	0	0	0	0	0	0	0	1	0
Australian Shepherd	3	0	0	0	0	2	0	0	0	0	1	0	0
Beagle	2	0	0	0	0	0	0	0	2	0	0	0	0
Bernese Mountain Dog	19	1	0	2	0	13	0	0	0	1	0	0	2
Bloodhound	1	0	0	0	0	1	0	0	0	0	0	0	0
Borzoi	1	0	0	0	0	1	0	0	0	0	0	0	0
Boxer	35	0	0	0	0	24	0	0	1	1	5	2	2
Caanan Dog	1	0	0	0	0	0	0	0	0	0	1	0	0
Cardigan Welsh Corgi	1	0	0	0	0	1	0	0	0	0	0	0	0
Cavalier King Charles Spaniel	2	0	0	0	0	1	0	0	0	0	0	1	0
Chesapeake Bay Retriever	6	0	0	2	0	3	0	0	0	0	0	1	0
English Cocker Spaniel	4	0	0	0	0	0	0	0	4	0	0	0	0
English Springer Spaniel	3	0	0	0	0	3	0	0	0	0	0	0	0
German Shepherd Dog	16	0	1	2	0	7	0	0	2	1	2	0	1
Golden Retriever	1	0	0	0	0	1	0	0	0	0	0	0	0
Kerry Blue Terrier	2	0	0	0	0	2	0	0	0	0	0	0	0
mixed breed	6	0	0	0	0	5	0	0	0	0	0	0	1
Pembroke Welsh Corgi	40	0	0	0	0	27	0	0	1	1	6	1	4
Pug	3	0	0	0	0	2	0	0	0	0	0	0	1
Rhodesian Ridgeback	11	0	0	1	0	5	0	0	1	0	3	0	1
Rottweiler	1	0	0	0	0	0	0	0	1	0	0	0	0
Rough Collie	1	0	0	0	0	1	0	0	0	0	0	0	0
Scottish Deerhound	1	0	0	0	0	0	0	0	1	0	0	0	0
Soft Coated Wheaten Terrier	2	0	0	0	0	2	0	0	0	0	0	0	0
Standard Poodle	2	0	0	0	0	1	0	0	1	0	0	0	0
Standard Schnauzer	1	0	0	0	0	0	0	0	1	0	0	0	0
Tibetan Terrier	2	0	0	0	0	0	0	0	0	0	2	0	0
White German Shepherd	1	0	0	0	0	0	0	0	0	0	1	0	0
Wire Fox Terrier	2	0	0	0	0	2	0	0	0	0	0	0	0
Total	173	1	1	8	0	105	0	0	15	4	21	6	12

"Pos" means aggregates containing SOD1 antigen were detected; "Neg" means aggregates containing SOD1 antigen were not detected.

6 of 18 spinal cords from dogs that were homozygous for the *SOD1:c.118A* allele had SOD1 aggregates and 4 of 25 spinal cords from dogs that were A/G heterozygotes at *SOD1:c.118* had SOD1 aggregates. These aggregates were not detected in any of the 15 spinal cords from control dogs that were homozygous for the *SOD1:c.118G* allele.

The occurrence of histopathologically confirmed DM in 8 dogs that were *A/G* heterozygotes at *SOD1:c.118* and had no other *SOD1* sequence variants prompted us to estimate the relative risks for developing DM for dogs with different *SOD1:c.118* genotypes (*A/A* versus *A/G* versus *G/G*). To avoid the large ascertainment biases associated with most of the genotypes in this report, we restricted our analysis to dogs that were clinically normal and <8 years old when samples were submitted for genotyping, but that were >10 years old when an update of their clinical status was requested from their owners in the form of an online survey. Dog owners responded by providing clinical updates for 137 dogs of 17 different breeds (Table S5). As indicated in Table 4, 18 of the 30 dogs with the homozygous *SOD1:c.118A* genotype developed clinical signs consistent with DM whereas only 2 of the 55 *SOD1:c.118A/G* heterozygous dogs and only 3 of the 52 *SOD1:c.118G* homozygous dogs developed compatible clinical signs.

Discussion

The DM-associated *SOD1:c.118A* allele appears to be widely distributed in the overall canine population. The allele was present in representatives from 124 different breeds or varieties, which accounts for 56% of the breeds represented in this study. For the 98 breeds in which only the *SOD1:c.118G* allele was detected, the mean number of genotyped dogs from each breed was only 23.4. Furthermore, in 49 of these breeds, <10 dogs were genotyped. The genotyping of more breed members would likely identify additional breeds harboring the *SOD1:c.118A* allele. The widespread distribution of this mutation among breeds suggests that it originated before the establishment of individual breeds. Because of the late onset of the disease signs associated with this mutation, there would be little natural selective pressure to decrease the frequency of the mutant allele. The breed-specific *SOD1:c.118A* allele frequencies varied greatly even among similar breeds. For example, all 53 of the genotyped Greater Swiss Mountain Dogs were homozygous for the ancestral *SOD1:c.118G* allele whereas the *SOD1:c.118A* allele frequency was 38% for the 2,413 genotyped representatives of the closely related Bernese Mountain Dog breed. Furthermore, even though before 1985 Smooth Fox Terriers and the Wire Fox Terriers were considered by the American Kennel Club to be varieties of a single breed which could be interbred, the *SOD1:c.118A* allele frequency was 94% for the 79 genotyped Wire Fox Terriers whereas all 30 Smooth Fox Terriers were *SOD1:c.118G* homozygotes. Because dogs were selected for genotyping for a variety of reasons, the breed-specific allele frequencies determined here are unlikely to reflect the precise overall allele frequencies for the various breeds.

In contrast to the widespread distribution of the *SOD1:c.118A* allele, the *SOD1:c.52T* allele appears to be restricted to Bernese Mountain Dogs. Even among the 912 genotyped Bernese Mountain Dogs, the *SOD1:c.52T* allele frequency was only 3.5%, much lower than the 38% *SOD1:c.118A* allele frequency in this breed. Of the 2 *SOD1:c.52T* homozygous Bernese Mountain Dogs, 1 developed DM as previously described[11] and the other was normal at the time of sample submission and is currently <8 years of age and thus younger than the expected age of onset for DM. The 24 Bernese Mountain Dogs that were heterozygous at both *SOD1:c.52* and *SOD1:c.118* were most likely compound heterozygotes. The development of signs of DM in 4 of these compound heterozygotes supports our earlier assertion that the *SOD1:c.52T* allele can cause or contribute to the development of DM.[11] We now know, however, that some *SOD1:c.118* heterozygous dogs with no other *SOD1* missense mutations have developed DM (see below). Documented development of DM in additional *SOD1:c.52T* homozygotes would provide more definitive evidence that the *SOD1:c.52T* allele can cause DM.

Crisp et al[15] have isolated detergent-insoluble SOD1 aggregates from the spinal cords of dogs with DM and shown that the amount of insoluble SOD1 is higher in spinal cords from more severely affected dogs. This confirms our earlier histopathologic demonstration of cytoplasmic SOD1 aggregate accumulation in the motor neurons from the spinal cords of DM-affected dogs.[4,11] Ogawa et al[16] have independently shown similarly appearing SOD1 aggregates in spinal cords from Pembroke Welsh Corgis with DM.

Table 4. Owner reported online survey results for dogs that were sampled when clinically normal and younger than 8 years old, but were 10 years old or older when their clinical status was updated.

Genotype at *SOD1:c.118*	No. of Dogs Queried	No. of Queries Answered	Percent Response	No. Without Clinical DM Signs	No. with Clinical DM Signs	Percent with Clinical DM Signs
G/G	204	52	25	49	3	6
A/G	177	55	31	53	2	4
A/A	131	30	23	12	18	60
Total	512	137	27	124	23	19

DM, degenerative myelopathy.

As shown in Table 3, cytoplasmic aggregates containing SOD1 antigen were found in spinal cord neurons in all but 1 of the examined dogs with histopathologically confirmed DM. The exception was a German Shepherd Dog without any sequence variants in SOD1 coding regions. Although human SOD1 was the first gene to be associated with amyotrophic lateral sclerosis (ALS),[17] mutations in many additional genes have been reported to cause ALS.[18] It seems likely that the DM in the German Shepherd Dog without SOD1 aggregates results from a distinct genetic or acquired etiology. Among the control dogs, cytoplasmic aggregates bound anti-SOD1 antibodies in spinal cord neurons from 6 of 18 SOD1:c.118A homozygotes and 4 of 25 SOD1:c.118A/G heterozygotes, even though the histopathologic examination of these cords did not support a diagnosis of DM. Mice overexpressing mutant human SOD1 transgenes have been commonly used as models to study ALS. These murine models develop SOD1-containing aggregates before their motor neuron deficits become apparent.[19] Thus, it seems plausible that some or all of the control dogs with cytoplasmic SOD1 aggregates were in a preclinical stage of DM when euthanized. Nonetheless, the occurrence of SOD1 aggregates in several of the control dogs indicates that their presence is not by itself a reliable diagnostic indicator for DM. In the previously mentioned transgenic mouse models, genetic background can affect neurodegeneration onset or progression.[20] This suggests that mean susceptibility to SOD1-associated DM could differ among breeds.

All of the dogs with confirmed DM in our earlier study were SOD1:c.118A homozygotes,[3] which suggested an autosomal recessive mode of inheritance. This differs from the autosomal dominant mode of inheritance typically seen with human SOD1-associated ALS.[21] We now report that the diagnosis of DM has been histopathologically confirmed in 8 dogs that were A/G heterozygotes at SOD1c.118, but had no other sequence variants predicted to alter the primary structure of the encoded SOD1. This finding is consistent with the hypothesis that the pathologic mechanisms underlying the development of DM are similar to the toxic gain-of-function mechanisms believed to be responsible for the development of SOD1-related ALS.[22] The occurrence of confirmed DM in these 8 SOD1c.118A/G heterozygotes suggested that compared to SOD1c.118G homozygotes, the heterozygotes are at increased risk of developing DM. This finding could call into question the strategy commonly used by dog breeders who mate SOD1:c.118A homozygotes or SOD1:c.118A/G heterozygotes only to SOD1:c.118G homozygotes to avoid the production of new generations of SOD1:c.118A homozygotes at risk of developing DM in old age. Thus, we wanted to estimate the risk of developing DM for dogs with the heterozygous SOD1c.118A/G genotype relative to SOD1:c.118A homozygotes and to SOD1:c.118G homozygotes. Most of our genotype results could not be used for this purpose because of the strong biases associated with sample acquisition. Instead, we estimated relative risks based only on the genotype results for clinically normal dogs that were sampled at <8 years of age but that were ≥10 years of age when their clinical status was recently updated. We considered the results from these dogs to have a relatively low ascertainment bias because this cohort of dogs was too young to have exhibited clinical signs of DM when the samples were submitted for genotyping. As indicated in Table S5, we received survey results updating the clinical status for 137 dogs from 17 breeds. Sixty percent of the 30 SOD1:c.118A homozygous dogs in this cohort developed clinical signs consistent with DM whereas only 4% of the 55 SOD1:c.118A/G heterozygotes and only 6% of the 53 SOD1:c.118G homozygotes developed clinical signs of DM (Table 4). This suggests that the relative risk of developing signs of DM for SOD1:c.118A/G heterozygotes is similar to that of G/G homozygotes and much lower than that for SOD1:c.118A homozygotes. Thus, the above-mentioned strategy of breeding to avoid the production of SOD1:c.118A homozygous puppies still appears to be warranted, particularly in breeds with high SOD1:c.118A allele frequencies where stricter selection strategies would come at the expense of selecting for other important traits.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. PCR primer sequences, amplicon sizes, and annealing temperatures for PCR amplification of canine *SOD1* exons.

Table S2. *SOD1:c.118* genotypes and *A* allele frequencies for individual breeds.

Table S3. *SOD1:c.52* genotypes and *T* allele frequencies for individual breeds or varieties.

Table S4. *SOD1:c.118* genotypes of DM-affected and control dogs with evaluated spinal cord histopathology.

Table S5. Compilation of owner-reported clinical-update survey results.

Fig S1. Photomicrographs of spinal cord sections with and without SOD1 aggregates.

Fig S2. DM Questionnaire.